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A low temperature embedding and section registration strategy for 3D image reconstruction of the rat brain from autoradiographic sections

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Abstract

In studies on animal models of human brain pathologies, three-dimensional reconstruction from histological sections is particularly useful when assessing the morphologic, functional and biochemical changes induced by pathology. It allows assessing lesion heterogeneity in planes different from the cutting plane and allows correlating the histology with images obtained in vivo, such as by means of magnetic resonance imaging. To create a 3D volume from autoradiographic sections with minimal distortion, both cryosectioning as well as section registration need to be optimal. This paper describes a strategy whereby four external fiducial markers are positioned outside the rat brain with the use of a low temperature brain embedding procedure. The fiducial markers proposed here can be rapidly added to any frozen tissue block with no impact on the subsequent histological operations. Since embedding is performed at a low temperature, no tissue degradation occurs due to sample heating. The markers enable robust and almost error free registration, even in the presence of missing sections and poor image quality. Furthermore, the markers may be used to partially correct for global distortions.

Keywords: Autoradiography; Histology; Matching; Marker; Cerebral blood flow; Alignment; Co-registration; Cryosections; External fiducial marker

1. Introduction

Cryosectioning techniques are used extensively to obtain unfixed sections of brain tissue for subsequent immunological, morphological, biochemical or autoradiographic analysis. While direct analysis of 2D histological sections is very useful, it may be of additional interest to digitally reconstruct a 3D histological volume (Nikou et al., 2003; Ourselin et al., 2001; Schormann and Zilles, 1998; Toga and Thompson, 2001). For this purpose, registration techniques are needed (Hill et al., 2001; Maintz and Viergever, 1998). Reconstruction of 3D histological volumes allows the investigation of morphologic, biochemical and functional changes in brain regions due to pathology. Furthermore, 3D reconstruction allows integration of information over multiple slices and it yields the possibility to extract information from an anatomical volume, which is intrinsically three-dimensional.

Since the introduction of non-invasive tomographic imaging modalities such as magnetic resonance imaging, computed tomography and positron tomography, there is also a great interest in spatially matching high resolution, ex vivo, histological volumes with lower resolution, in vivo, images to assess the relationship between measured signal, micro-anatomy and function of the brain (Humm et al., 2003; Mega et al., 1997). This comparison involves the registration of two 3D volumes, from which at least one has been obtained from a set of 2D sections (Malandain et al., 2004).

Quality of the 3D volume reconstructed from a set of 2D sections is highly dependent on the registration method applied and on the quality of the individual sections. In this study, the 2D brain sections were obtained from rats that had been injected a freely diffusible radioactive tracer into the blood stream to quantify local cerebral blood flow by means of a classical autoradiographic approach (Sakurada et al., 1978).

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Registration methods based on the use of intrinsic landmarks (Maintz and Viergever, 1998) are not satisfactory in the case of autoradiography due to the time lapse between animal sacrificing and brain freezing. This causes the images to be unsharp (broadening of the point spread function), and therefore it becomes difficult to find valid intrinsic markers. When images are registered sequentially with respect to the preceding one, and if the number of slices is large, a global volume distortion may occur due to error propagation (Hess et al., 1998; Nikou et al., 2003). A basic problem in image registration is that consecutive slices are naturally dissimilar, even in the absence of data corruption. Finally, the non-contiguous sampling of images induces global deformations due to the shape of the object (so-called "banana problem") (Malandain et al., 2004; Streicher et al., 1997). These registration difficulties can be avoided by using extrinsic markers, either inside or outside the tissue sample. Accurately positioning internal markers in frozen tissue is difficult and will induce tissue damage (Hess et al., 1998).

During cryosectioning, brain slices may be deformed due to tears and folds. Some sections are so strongly affected that they may have to be discarded, leading to missing data. Also, compression of the section may occur due to the pressure of the cutting blade. The eventual distortion is dependent on the cutting depth, the rigidity of the material and the operator's skill (Zarow et al., 2004). These difficulties could be partially avoided by embedding the brain before cutting.

Brain embedding provides the opportunity to add fiducial markers outside the organ, and thus to perform extrinsic registration without inducing tissue damage. Classical embedding methods, whether applied with or without external markers, are performed at room, or even at higher temperatures (Bjarkam et al., 2001; Sorensen et al., 2000; Streicher et al., 1997; Williams and Doyle, 1996). They cannot be applied to frozen tissues, in particular to autoradiographic sections that may not defrost during processing.

To reconstruct 3D autoradiography volumes from tumour bearing rat brains with minimal distortion due to sectioning and registration, we developed a low temperature embedding procedure that includes the placement of four external fiducial markers. The procedure was optimized to embed the frozen brain at the lowest temperature possible. This is of particular importance in studies where diffusible tracers are used. The fiducial markers are clearly visible at the corners of the sections eventually obtained. Their positions are readily identified following segmentation of the digitized sections. These positions may be used to calculate the rotation/translation matrices that optimally register the entire set of sections. Moreover, the proposed approach can be performed rapidly (within less than 30 min) and has no impact on the earlier or later stages of the histological protocol.

2. Materials and methods

2.1. Animal handling

All operative procedures and animal care strictly conformed to French government guidelines (licenses 380321, A3851610004 and B3851610003). A total of four adult male Wistar rats (299 ± 35 g) were used in this study. For tumour implantation, rats were anesthetized (400 mg/kg chloral hydrate) and placed on a stereotactic head holder. A scalp incision was performed along the median line. A 2.5-mm diameter burr hole was drilled in the skull 3.5 mm lateral to the bregma. The cell suspension (10^5 cells in 5 μl) was injected in 10 s into the right caudate nucleus, at a depth of 3.5 mm under the dura. The Hamilton syringe was slowly removed 1 min after the injection. The burr hole was plugged with Horsley wax and the scalp sutured. Three weeks after tumour implantation, rats were anesthetized (isoflurane 4%) and maintained under a mixture of isoflurane (1.5%), air and O_2 ($FiO_2 = 40\%$). Arterial and venous catheters were placed in the femoral vessels. Rectal temperature was maintained at $37.0 \pm 0.5\,^{\circ}C$ throughout the experiment with a feedback-controlled heating pad.

2.2. Autoradiography

Autoradiography was performed following the procedure described by Sakurada et al. (1978). In rats, approximately 30 μCi of iodo[^{14}C]antipyrine in 1.2 ml of normal saline was infused during 1 min via a femoral venous catheter. The infusion rate was steadily increased from 1 to 1.5 ml/min. At the end of infusion, the animal was decapitated. The brain was rapidly dissected out and frozen in liquid nitrogen.

2.3. Embedding strategy

For embedding and subsequent cryosectioning of the frozen brain, we used an aluminium container (Fig. 1). The latter was mounted on a support and consists of several parts: a chuck, a square tube made of two parts screwed together, a lid and four needles of 1 mm diameter and 31 mm of length. The chuck is 22 mm × 18 mm, and has a crossing grid pattern to optimize gripping of the embedded tissue block. The tube is 35 mm high and fits over the chuck. The lid and the chuck have four holes each (1 mm in diameter) positioned in the corners at 2.5 mm from the edges and a large hole in the middle. Each needle is therefore guided through two holes, one in the lid and one in the chuck. This ensure that, when inserted, the needles are perfectly perpendicular to the chuck, and therefore to the cutting plane. All parts are stored at $-25\,^{\circ}C$.

The following procedure is followed for brain embedding. The chuck is mounted on the support. At each corner of the chuck (2.5 mm from the sides) a hole of 1 mm in diameter makes it possible to mount plastic spacers (Ø 6 mm, 16 mm height). These spacers facilitate a more precise and reproducible placement of the brain (Fig. 1a) and ensure that the brain will be sufficiently separated from the needles at their insertion. The brain is glued onto the chuck with Neg-50® (Richard Allan Scientific) stored at $4\,^{\circ}C$ using a cooled pair of tweezers. The plastic spacers are then removed and the tube is placed over the chuck. The lid is then put in place and the needles are inserted into the four corner holes (Fig. 1b). All operations are performed inside a cryotome at $-25\,^{\circ}C$. Next, Neg-50® at $4\,^{\circ}C$ is poured quickly into the container through the central hole in the lid. To reach the